

Chem 4540 Enzymology Winter 2005
Problem Set #2 ANSWERS

1. Give the systematic names and the full E.C. classifications of the enzymes catalysing the following reactions: (note that you will need to access the Enzyme Nomenclature web site which has a link found on the Course Website in order to find the serial number). The URL for this site is <http://www.expasy.ch/enzyme/>.

(a)
$$\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-\text{CH}_2-\text{CH}_2-\overset{+}{\text{N}}(\text{CH}_3)_3 + \text{H}_2\text{O} \rightleftharpoons \text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}^- + \text{HOCH}_2-\text{CH}_2-\overset{+}{\text{N}}(\text{CH}_3)_3$$
 acyl choline acid anion choline
 acylcholine: acyl
 hydrolase
 (3.1.1.8)
 Cholinesterase

(b)
$$\text{H}_2\text{N}-\overset{\text{O}}{\parallel}{\text{C}}-\text{OPO}_3^{2-} + \text{H}_3\overset{+}{\text{N}}(\text{CH}_2)_3-\overset{+}{\text{N}}\text{H}_3-\text{CH}-\text{CO}_2^- \rightleftharpoons \text{H}_2\text{N}-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}(\text{CH}_2)_3-\overset{+}{\text{N}}\text{H}_3-\text{CH}-\text{CO}_2^- + \text{P}_i$$
 carbamoyl phosphate L-ornithine citrulline
 Carbamoyl phosphate:
 L-ornithine carbamoyl
 transferase
 (2.1.3.3) Ornithine carbamoyl
 transferase

(c)
$$\text{ATP} + \text{H}_3\overset{+}{\text{N}}-\underset{\text{CH}_3}{\text{CH}}-\text{CO}_2^- + \text{H}_3\overset{+}{\text{N}}-\underset{\text{CH}_3}{\text{CH}}-\text{CO}_2^- \rightleftharpoons \text{H}_3\overset{+}{\text{N}}-\underset{\text{CH}_3}{\text{CH}}-\overset{\text{H}}{\parallel}{\text{C}}-\underset{\text{CH}_3}{\text{N}}-\underset{\text{CH}_3}{\text{CH}}-\text{CO}_2^- + \text{ADP} + \text{P}_i$$
 D-alanine D-alanine D-alanyl-alanine
 D-alanine: D-alanine Ligase
 (ADP-forming) (6.3.2.4)

(d)
$$\begin{array}{c} \text{CH}_2\text{OH} \\ | \\ \text{C}=\text{O} \\ | \\ \text{CH}_2\text{OH} \end{array} + \text{NADH} + \text{H}^+ \rightleftharpoons \begin{array}{c} \text{CH}_2\text{OH} \\ | \\ \text{CHOH} \\ | \\ \text{CH}_2\text{OH} \end{array} + \text{NAD}^+$$
 dihydroxyacetone glycerol
 glycerol: NAD⁺ 2-oxidoreductase
 (1.1.1.6) glycerol dehydrogenase
 (Such reactions are classified in the direction where NAD⁺ is the electron acceptor rather than NADH the electron donor; by convention)

(e)
$$\begin{array}{c} \text{O} \\ || \\ \text{C}-\text{NH} \end{array} + \text{HCO}_3^- \rightleftharpoons \text{CO}_2 + \text{H}_2\text{NCOO}^-$$
 cyanate bicarbonate carbamate
 cyanate: carbamate
 lyase (4.3.99.1)
 cyanate lyase

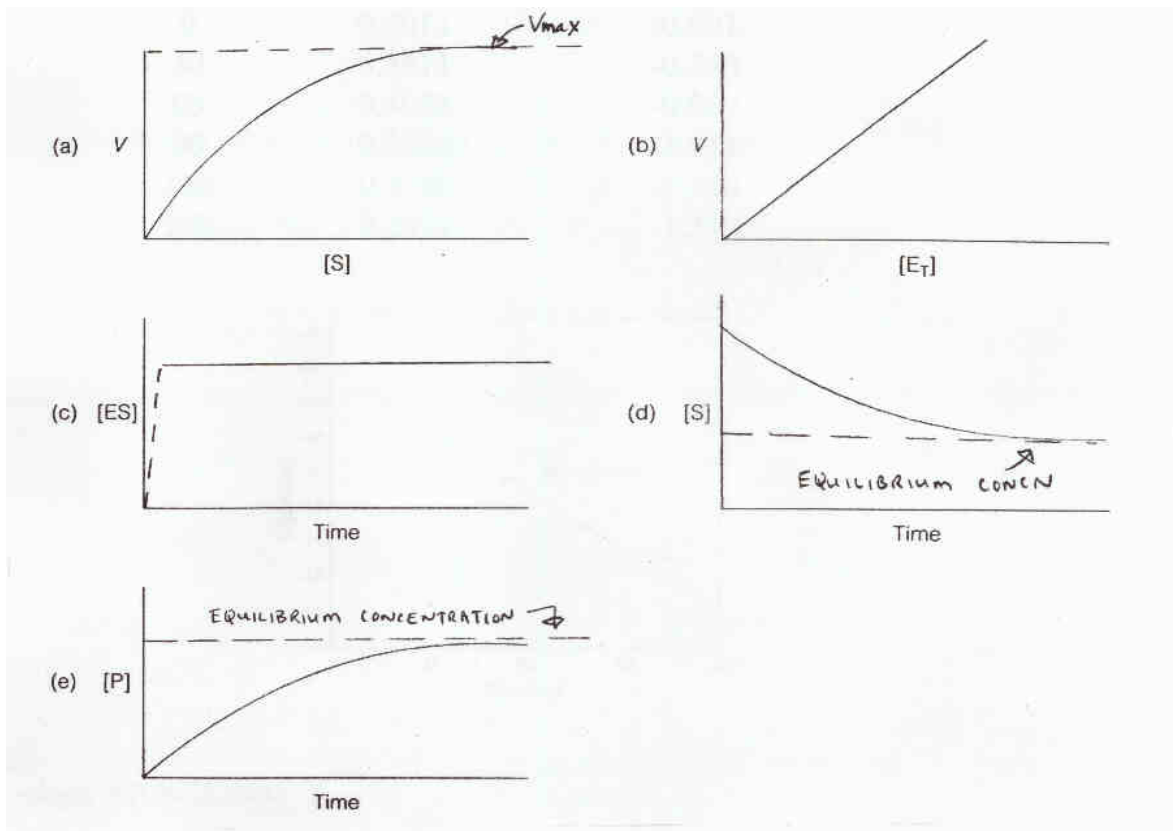
(f)
$$\text{NADH} + 2 \text{ ferri cytochrome } b_5 \rightleftharpoons \text{NAD}^+ + 2 \text{ ferro cytochrome } b_5$$
 cytochrome b₅ reductase

(g)
$$\text{UDP-galactose} \rightleftharpoons \text{UDP-glucose}$$
 2-ferrocytochrome b₅: NAD⁺ oxidoreductase (1.6.2.2)
 (glucose and galactose are aldohexoses differing in configuration at C4).

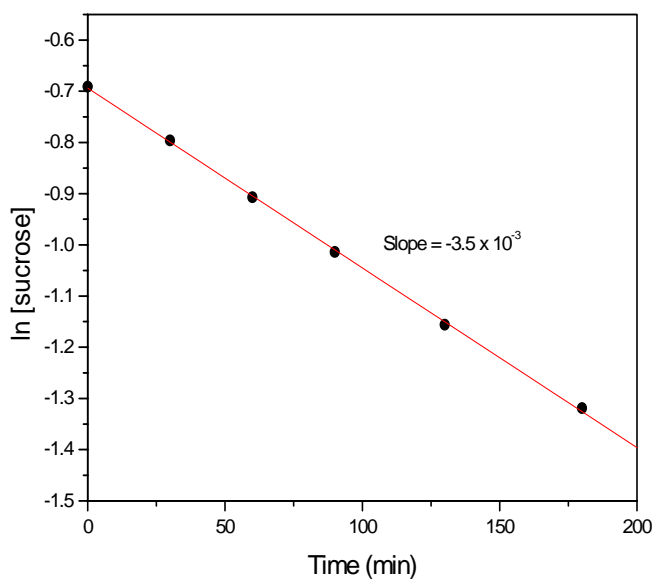
UDP-galactose: 4-epimerase (5.1.3.2)

2. **Web-based exercise.** (a) 5- β -D-Ribofuranosylpicolinamide Adenine Dinucleotide (Cpad) is a substrate analogue (for NAD^+) for the enzyme that binds like NAD^+ but the redox reaction can not occur in its presence. (b) The file was deposited on Dec 13, 1993. The resolution for the structure is 2.70 Ångstroms? There are two subunits in the x-ray structure of the alcohol dehydrogenase protein. The heterogeneous compounds associated with the protein include CPAD, ethanol, and two Zn^{2+} ions. (c) The molecular weight of each subunit is 39791 D and each includes 374 residues, 10 alpha helices, and 19 beta strands. There is a difference in the secondary structure between the two residues likely because the dimerization process has induced this structural change in one of the monomers. (d) Now select “Down/Display File” and choose a PDB file to download (select the uncompressed PDB file). Save the file to an appropriate folder on your computer desktop (note the name of the pdb file). **Optional activity.** Check the course website and click on LADH Rasmol File to view my rendition of the dimeric alcohol dehydrogenase enzyme from *Equus Caballus*.

3. Sketch the appropriate plots on the following axes. Assume that simple Michaelis-Menten kinetics apply.



4.	Time (min)	[sucrose] M	ln[sucrose]
	0	0.5011	-0.691
	30	0.4511	-0.796
	60	0.4038	-0.907
	90	0.3626	-1.014
	130	0.3148	-1.156
	180	0.2674	-1.319



$$k = -\text{slope} = 0.0035 \text{ min}^{-1}$$

$$t_{1/2} = 0.693/k = 0.693/0.0035 \text{ min}^{-1} = 198 \text{ min}$$

The reaction follows *pseudo* first-order kinetics because in aqueous solution the H_2O concentration of 55.5 M remains essentially constant in comparison to the sucrose concentration. The reaction actually is second order.

$$(1-0.99) = 0.01 = (1/2)^n \text{ where } n \text{ is the number of half-lives}$$

$$n (\log 0.5) = \log 0.01$$

$$n = -2.00/-0.301 = 6.64 \text{ half-lives. Thus, the time to hydrolyze 99\% of the sucrose} = t_{0.01} = nt_{1/2} = 6.64 \times 198 = 1315 \text{ min.}$$

The time to react a given fraction of a substance that reacts in a first-order process is independent of the amount initially present. Hence, the time to hydrolyze 99% of 2×0.5011 M sucrose is also 1315 min.

5. (a) $[\text{S}]_0 = 0.5 \text{ } \mu\text{mole}/25 \text{ mL} = 2 \times 10^{-2} \text{ } \mu\text{mole}/\text{mL} = 2 \times 10^{-5} \text{ M}$
 $K_M = 6 \times 10^{-4} \text{ M}$ therefore $[\text{S}]_0 < K_M$ and thus **first order** kinetics.

(b) $[\text{S}]_0 = 0.5 \text{ mmole}/25 \text{ mL} = 2 \times 10^{-2} \text{ mmole}/\text{mL} = 2 \times 10^{-2} \text{ M}$
 $K_M = 6 \times 10^{-4} \text{ M}$ therefore $[\text{S}]_0 > K_M$ and thus **zero order** kinetics.

6. (a) We must assume that the initial velocity observed at the $[\text{S}] = 0.01 \text{ M}$ is the V_{max} , i.e., $[\text{S}]_0$

$\gg K_M$, therefore $v_0 = V_{\max} = 35 \mu\text{mole L}^{-1}\text{min}^{-1}$.

At $[S]_0 = 3.5 \times 10^{-5}\text{M}$ then $v_0 = (35 \mu\text{mole L}^{-1}\text{min}^{-1}) (3.5 \times 10^{-5}\text{M}) / [(2.5 \times 10^{-5}\text{M}) + (3.5 \times 10^{-5}\text{M})] = 22.3 \mu\text{mole L}^{-1}\text{min}^{-1}$.

(b) At $4.0 \times 10^{-4}\text{M}$ $[S]_0$, $[S]_0$ is between K_M and $100 K_M$ (note: at $[S]_0 \geq 100K_M$, $v_0 = V_{\max}$)

Thus, v_0 will be between $17.5 \mu\text{mole L}^{-1}\text{min}^{-1}$ ($V_{\max}/2$) and $35 \mu\text{mole L}^{-1}\text{min}^{-1}$ (V_{\max})

$v_0 = (35 \mu\text{mole L}^{-1}\text{min}^{-1}) (4 \times 10^{-4}\text{M}) / [(2.5 \times 10^{-5}\text{M}) + (4 \times 10^{-4}\text{M})] = 33.3 \mu\text{mole L}^{-1}\text{min}^{-1}$.

(c) At $2 \times 10^{-4}\text{M}$ $[S]_0$ then $[S]_0 = (10) K_M$

$v_0 = V_{\max}[S]/(K_M + [S]) = V_{\max}(10 K_M)/(K_M + 10 K_M) = V_{\max}(10/11) = 0.91 V_{\max}$

$v_0 = 0.91(35 \mu\text{mole L}^{-1}\text{min}^{-1}) = 31.9 \mu\text{mole L}^{-1}\text{min}^{-1}$.

(d) At $2 \times 10^{-6}\text{M}$ $[S]_0$ then $[S]_0 = 0.1 K_M$

$v_0 = V_{\max}(0.1 K_M)/(K_M + 0.1 K_M) = V_{\max}(0.1 K_M)/1.1 K_M = 0.091 V_{\max}$

$v_0 = 0.091(35 \mu\text{mole L}^{-1}\text{min}^{-1}) = 3.19 \mu\text{mole L}^{-1}\text{min}^{-1}$.

(e) At $1.2 \times 10^{-6}\text{M}$ $[S]_0$: $v_0 = (35 \mu\text{mole L}^{-1}\text{min}^{-1}) (1.2 \times 10^{-6}\text{M}) / [(2 \times 10^{-5}\text{M}) + (1.2 \times 10^{-6}\text{M})] = 1.98 \mu\text{mole L}^{-1}\text{min}^{-1}$.

7. $v_0/V_{\max} = [S]/(K_M + [S]) \quad 0.90/1 = [S]_{90}/(K_M + [S]_{90})$

$0.90 K_M + 0.90[S]_{90} = [S]_{90} ; 0.90K_M = [S]_{90} - 0.90[S]_{90};$

$0.90K_M = 0.10 [S]_{90}; [S]_{90} = 0.90K_M/0.10; [S]_{90} = 9K_M$

Thus, when the substrate concentration is $9K_M$, the v_0 is 90% of the V_{\max} .

$v_0/V_{\max} = [S]/(K_M + [S]) \quad 0.10/1 = [S]_{10}/(K_M + [S]_{10})$

$0.10K_M + 0.10[S]_{10} = [S]_{10} ; 0.10K_M = [S]_{10} - 0.10[S]_{10};$

$0.10K_M = 0.90 [S]_{10}; [S]_{10} = 0.10K_M/0.90; [S]_{10} = 0.111K_M$

Thus, when the substrate concentration is $0.111K_M$ then $v_0 = 10\%$ of V_{\max} .

$[S]_{90}/[S]_{10} = 9K_M/0.111K_M = 81$

8. (a) $[S]_0 = 2 \times 10^{-5}\text{M} \ll K_M$ therefore reaction is first order w.r.t. substrate concn.

For a first order reaction: $t_{1/2} = 0.693/k$, so $6 \text{ min} = 0.693/k$, $k = 1.16 \times 10^{-1} \text{ min}^{-1}$.

(b) Recall from lecture notes: $k = V_{\max}/K_M$ for a first order reaction so then $V_{\max} = k(K_M)$

$V_{\max} = (1.16 \text{ min}^{-1})(5 \times 10^{-3}\text{M}) = 5.8 \times 10^{-4} \text{ M min}^{-1}$

(c) For a first order reaction: $2.303 \log([S]_0/[S]_t) = kt$ (integrated Michaelis-Menten equation).

$2.303 \log (2 \times 10^{-5} \text{ M}/[S]_t) = 0.116 \text{ M min}^{-1}(15 \text{ min})$

$2.303 (\log 2 \times 10^{-5} - \log [S]_t) = 1.733 \text{ M}$

$\log 2 \times 10^{-5} - \log [S]_t = 0.752 \text{ M}$

$(0.301 - 5) - \log [S]_t = 0.752 \text{ M}$

$-\log [S]_t = 5.451 \text{ M}$

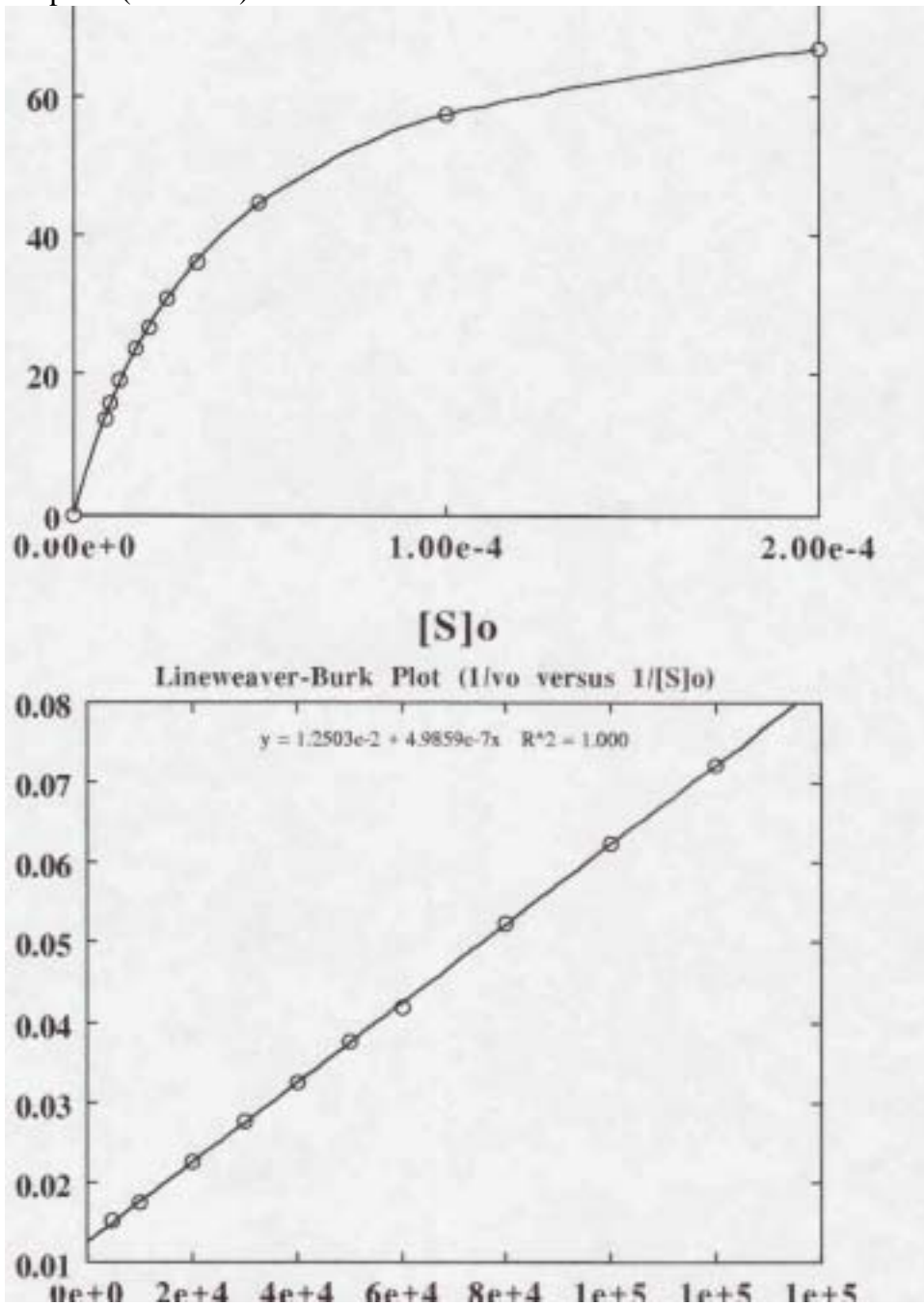
$1/[S]_t = 2.825 \times 10^5$

$$[S]_t = 3.54 \times 10^{-6} \text{ M}$$

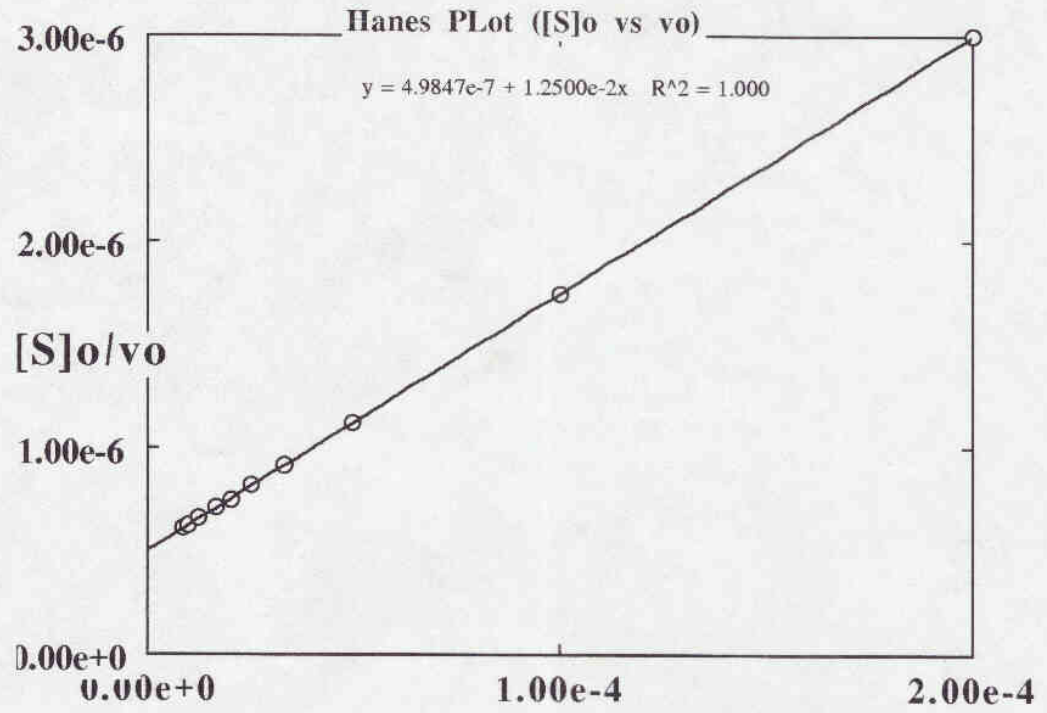
$$\text{Thus, } [P]_{15} = [S]_0 - [S]_t = (2 \times 10^{-5} \text{ M}) - (3.54 \times 10^{-6} \text{ M}) = 1.646 \times 10^{-5} \text{ M}$$

9. (a) See graph 9(a) Lineweaver-Burk plot, $y = 1.2503 \times 10^{-2} + 4.986 \times 10^{-7} X$
Y intercept = $1/V_{\max} = 1.25 \times 10^{-2}$ $V_{\max} = 80 \text{ } \mu\text{moles L}^{-1}\text{min}^{-1}$
X intercept = $-1/K_M = -1.25 \times 10^{-2}/4.986 \times 10^{-7} = -2.51 \times 10^4$
 $K_M = -(1/-2.51 \times 10^4) = 4.0 \times 10^{-5} \text{ M}$
- (b) See graph 9(b) Hanes Plot, $y = 4.986 \times 10^{-7} + 1.25 \times 10^{-2} X$
Slope = $1.25 \times 10^{-2} = 1/V_{\max} = 1/1.25 \times 10^{-2} = 80 \text{ } \mu\text{moles L}^{-1}\text{min}^{-1}$
Y intercept = $K_M/V_{\max} = 4.986 \times 10^{-7}$, $K_M = 80(4.986 \times 10^{-7}) = 3.99 \times 10^{-5} \text{ M}$
- (c) See graph 9(c) Eadie-Hofstee Plot, $y = -3.992 \times 10^{-5} X + 80.04$
Slope = $-K_M = -(-3.992 \times 10^{-5}) = 3.99 \times 10^{-5} \text{ M}$
Y intercept = $V_{\max} = 80 \text{ } \mu\text{moles L}^{-1}\text{min}^{-1}$
- (d) See graph 9(d), $y = 2.01 \times 10^6 - 2.51 \times 10^4 X$ Eadie-Scatchard Plot
Slope = $-1/K_M = -1/2.50 \times 10^4 = 4.0 \times 10^{-5} \text{ M} = K_M$
Y intercept = $V_{\max}/K_M = 2.01 \times 10^6$, $V_{\max} = 2.01 \times 10^6 (4.0 \times 10^{-5}) = 80 \text{ } \mu\text{moles/L}^{-1} \text{ min}^{-1}$
- (e) See graph 9(e), $V_{\max} = 78 - 82 \text{ } \mu\text{moles L}^{-1}\text{min}^{-1}$; $K_M = 3.6 - 4.4 \times 10^{-5} \text{ M}$.

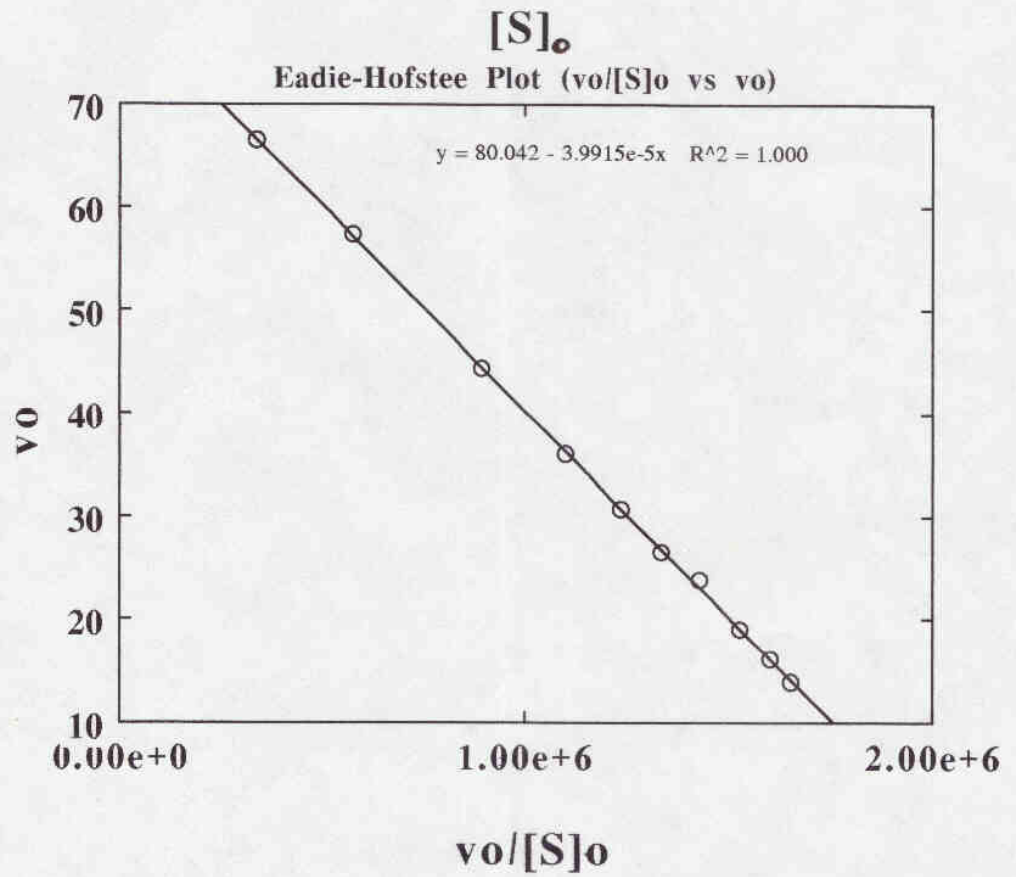
Graphs 9 (ai and aii)



(b)

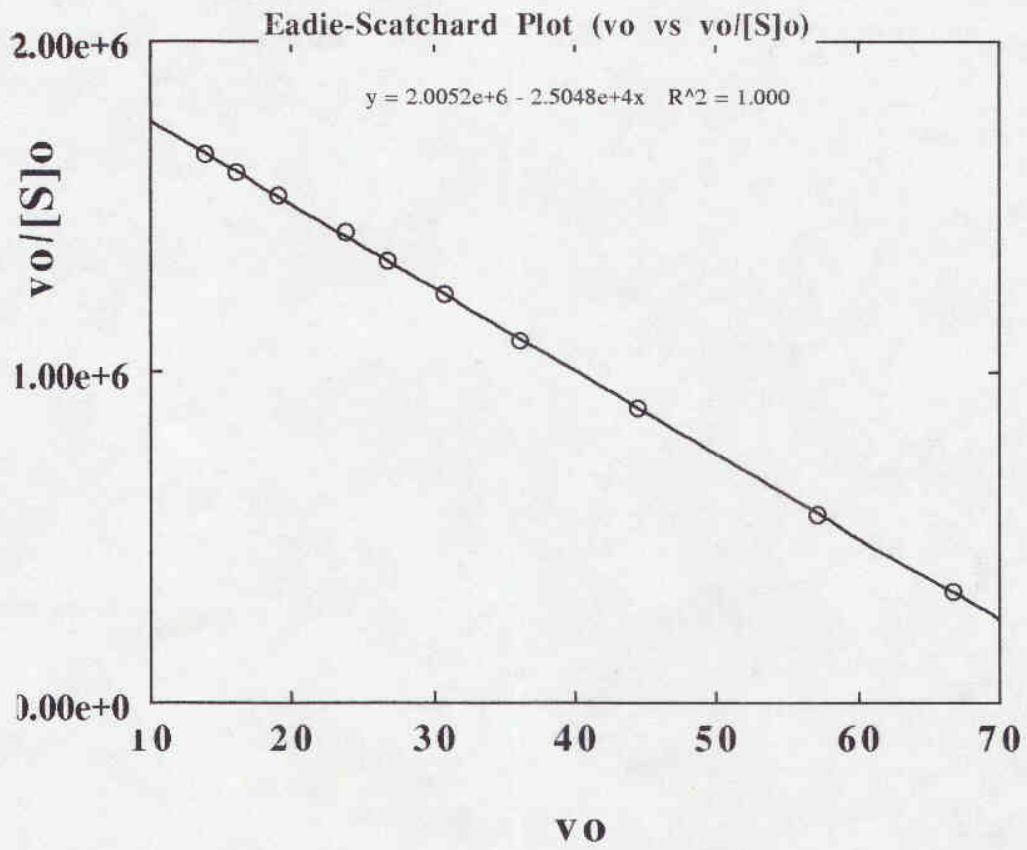


(c)



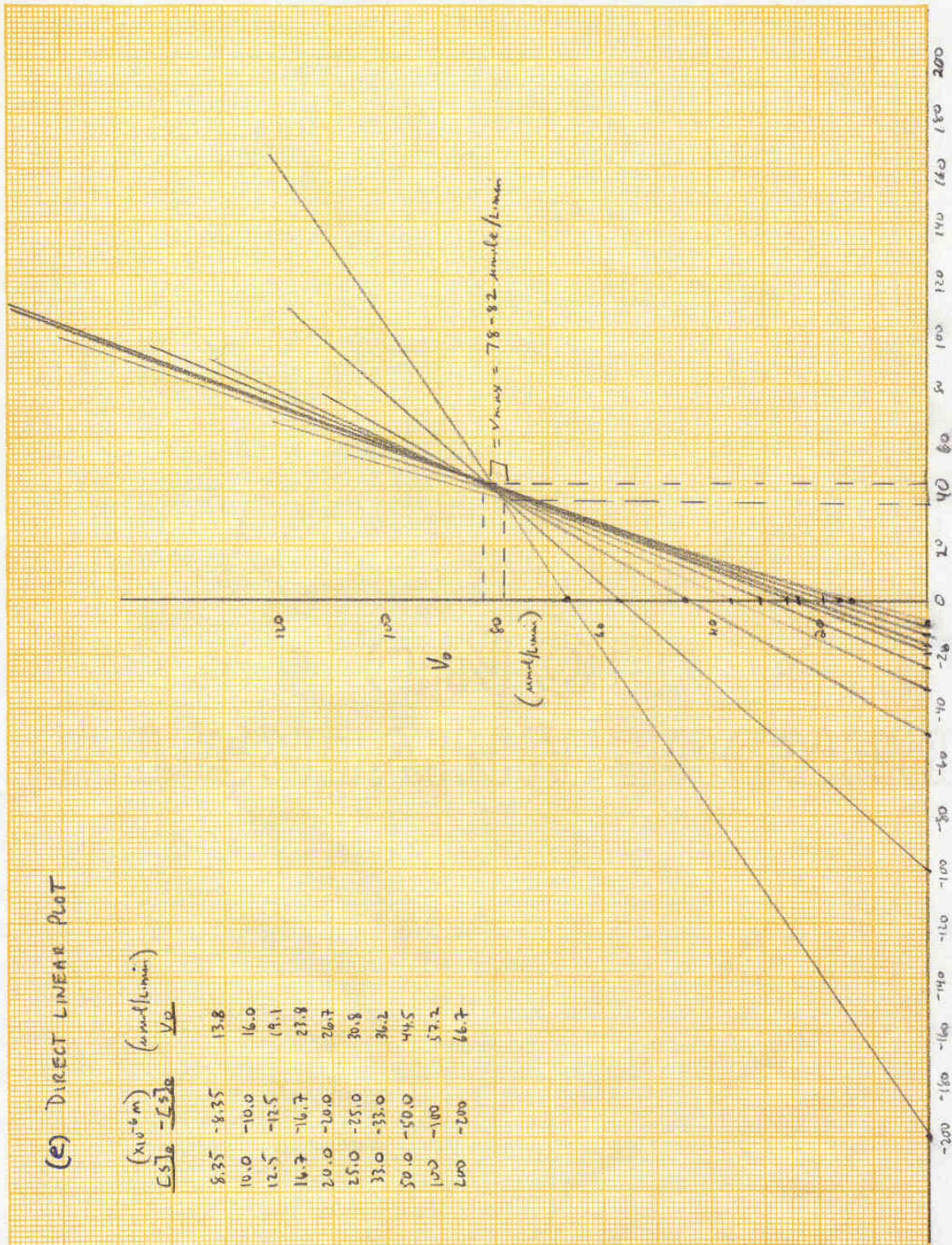
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GRAPHS

(d)



(e) DIRECT LINEAR PLOT

$(X \times 10^6 M)$ $CS]_0 = [S]_0$	$(\text{min}/\text{L/min})$ V_0
8.35	13.8
10.0	16.0
12.5	19.1
16.7	23.9
20.0	26.7
25.0	30.8
33.0	36.2
44.5	44.5
57.5	52.2
60.2	66.7



$CS]_0 \times 10^{-6} M$

$CS]_0 \times 10^{-6} M$

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10. (a) $[S]_0 = 1 \times 10^{-5} \text{M} \ll K_M (2 \times 10^{-3} \text{M})$ therefore first order and the reaction will be directly proportional to $[S]$. Since $[S]$ decreases with time the velocity will also decrease with time. For first order reactions, a constant proportion (not amount) is converted to product per unit time.
- $$2.303 \log [S]_0/[S]_t = kt$$
- Firstly, calculate k knowing that at the end of 1 minute 98% of the original substrate remains.
- $$2.303 \log(100/98) = k(1\text{min})$$
- $$2.303 \log (1.02) = k \text{ min}$$
- $$k = 1.98 \times 10^{-2} \text{ min}^{-1}$$
- Next, calculate $[S]_t$ at $t = 3 \text{ min}$
- $$2.303 \log(100/[S]_t) = 1.98 \times 10^{-2} \text{ min}^{-1}(3 \text{ min})$$
- $$2.303(\log 100 - \log [S]_t) = 5.94 \times 10^{-2}$$
- $$4.606 - 2.303 \log [S]_t = 5.94 \times 10^{-2}$$
- $$-2.303 \log [S]_t = -4.547$$
- $$\log [S]_t = 1.974$$
- $$[S]_t = 94.2\% \text{ of original } [S]$$
- $$[S]_t = 1 \times 10^{-5}(0.942) = 9.42 \times 10^{-6} \text{ M.}$$
- Therefore, the $[P]$ will be $100 - 94.2 = 5.8\% = 1 \times 10^{-5} \text{M} (0.058) = 5.8 \times 10^{-7} \text{ M.}$
- (b) If $[S] = 10^{-6} \text{ M}$ the reaction is still first order. The proportion of substrate converted to product would still be 5.6% by 3 min. However, the absolute amount of substrate consumed (and product formed) would obviously be less than in part (a) above.
- (c) V_{\max} can be estimated since we know K_M (hence the reaction order)
- At $[S]_0 = 1 \times 10^{-5} \text{ M}$ the velocity for the first minute is : $v_0 = k[S]$
- $$v_0 = 0.02 \text{ min}^{-1}(1 \times 10^{-5} \text{M}) = 2 \times 10^{-7} \text{M min}^{-1}$$
- $$v/V_{\max} = [S]/(K_M + [S])$$
- $$2 \times 10^{-7} \text{M min}^{-1}/V_{\max} = 1 \times 10^{-5} \text{M}/(2 \times 10^{-3} \text{M}) + (1 \times 10^{-5} \text{M})$$
- $$V_{\max} = [(2 \times 10^{-3} + 1 \times 10^{-5})(2 \times 10^{-7})/(1 \times 10^{-5})] \text{ M min}^{-1} = 4.02 \times 10^{-5} \text{ M min}^{-1}$$
- (d) V_{\max} will be observed at about $100 K_M$ (rule of thumb estimate) therefore $100(K_M) = 100(2 \times 10^{-3} \text{M}) = 2 \times 10^{-1} \text{M} = 0.2 \text{ M}$
- (e) At $0.2 \text{M } [S]_0$ the reaction will be zero order
- $$[P] = V_{\max}(t) = (4.02 \times 10^{-5} \text{ M min}^{-1})(3 \text{ min}) = 12.06 \times 10^{-5} \text{ M.}$$
- $$12.06 \times 10^{-5} \text{ M}/2 \times 10^{-1} \text{M} (100) = 6.03 \times 10^{-2} \% \cong 0.06\%.$$
11. Lineweaver-Burk equation: $(1/v_0) = K_M/V_{\max}(1/[S]_0) + 1/V_{\max}$
- $$\text{Slope} = K_M/V_{\max} = 2.5 \times 10^{-4} \text{ min}$$
- $$\text{Y intercept} = 1/V_{\max} = 3.0 \times 10^{-2} \text{ M}^{-1} \text{ min}$$
- $$V_{\max} = 33.33 \text{ M min}^{-1} \text{ then}$$
- $$K_M = (33.33 \text{ M min}^{-1})(2.5 \times 10^{-4} \text{ min}) = 8.33 \times 10^{-3} \text{M.}$$
12. (a) $v_0 = V_{\max}[S]/(K_M + [S])$ $v_0/V_{\max} = [S]/(K_M + [S])$ $v_0/V_{\max} = 1/(10 + 1) = 1/11 = 0.091$
- (b) $v_0/V_{\max} = 10/(1 + 10) = 10/11 = 0.909.$